USE OF HIGH MOLECULAR WEIGHT BIOPOLYMERS TO IMPROVE THE PROPERTIES OF CHROME-FREE LEATHER*

by

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ABSTRACT

In prior studies, we addressed the problems of poor leather quality, specifically "spring break" hides, by utilizing fillers produced from enzymatically-modified waste proteins, in particular those proteins from the leather and dairy industry (low quality gelatins and caseins or whey). In a more recent study, we applied low molecular weight enzymatically-modified gelatin/whey protein isolate (WPI) fillers to chrome-free or "wet white" leather. The leather was subsequently evaluated and it was found, as seen in previous studies the mechanical properties of the treated leather were not significantly different from controls, but there was an improvement in the subjective properties, specifically in fullness. In this present study, we attempted not only to further improve subjective properties of chrome-free leather, but also to use a more economical whey protein concentrate (WPC)/gelatin combination as well as substituting sodium bisulfite ($NaHSO_3$) for the more expensive dithiothreitol (DTT), which is used to denature whey prior to enzyme modification. High molecular weight products were prepared from enzymatically-modified WPC/gelatin, whose properties could be described as having high viscosities and melting points and whose SDS-PAGE gels showed that the WPC and gelatin had been extensively modified. These products were applied to chrome-free leather and subjective properties, in particular fullness, break, and overall evaluation, were better than seen previously. Furthermore, when $NaHSO_3$ was substituted for dithiothreitol (DTT), products with a reproducible range of physical properties could be realized. Thus, by utilization of higher molecular weight WPC/gelatin products on chrome-free leather, we have not only made treatments that are more economical with subsequent improvement in leather quality but we also enhanced product preparation by utilization of $NaHSO_3$ to denature the whey protein.

RESUMEN

En estudios anteriores, nos enfocamos en los problemas de baja calidad del cuero, específicamente en pieles de la estación primaveral, con su característica flor suelta, utilizando rellenantes producidos con desperdicios proteínicos enzimáticamente modificados, originados desde las industrias del cuero y lácteos (gelatinas de baja calidad y caseína o suero). En un estudio más reciente, ofrecimos rellenantes de bajo peso molecular originados de proteínas provenientes de gelatina/suero lácteo (WPI) modificadas enzimáticamente, aplicadas a cueros exentos de cromo o sea "wet-white". El cuero fue subsecuentemente evaluado y se encontró, tal como visto en estudios anteriores, que las propiedades físicas del cuero así tratado aunque no eran superiores a las de los cueros controles, sin embargo hubo una mejoría en las propiedades subjetivas, especialmente en la llenura. En este presente estudio, intentamos no solo incrementar las propiedades subjetivas del cuero exento de cromo, así cómo el utilizar una combinación más económica del los concentrados proteínicos provenientes del suero lácteo (WPC)/ gelatina por medio de la sustitución por bisulfito de sodio ($NaHSO_3$) para los más caros productos basados en ditiotreitol (DTT), que se emplean para desnaturalizar el suero lácteo antes de su modificación enzimática. Productos de altos pesos moleculares fueron así preparados con WPC/gelatina enzimáticamente modificada, cuyas resultantes propiedades podrían describirse como exhibiendo altas viscosidades y temperaturas superiores de fusión y cuyos análisis por electroforesis en geles (SDS-PAGE) demostraron que la WPC y gelatina habían sido extensivamente modificadas. Estos productos fueron empleados en cuero exento de cromo y las cualidades más subjetivas, principalmente llenura, firmeza de flor y aspecto en general, se vieron mejoradas en comparación con los

*Presented in part at the 106th annual American Leather Chemists Association Meeting, June 10-13, 2010, Grand Geneva, Lake Geneva, WI

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Manuscript received July 20, 2011, accepted for publication July 28, 2011

resultados anteriores. Más aún, cuando $NaHSO_3$ sustituyó a los productos con ditiotreitol (DTT), lo cual resultó en un rango de cuero más reproducible en cuanto a propiedades físicas. Entonces, por utilización de productos rellenantes de más peso molecular, basado en WPC/gelatina, para cuero exento de cromo, no solo habremos logrado tratamientos más económicos acompañados de mejoría en la calidad del cuero, pero también alcanzando una preparación superior por el uso del $NaHSO_3$ en la desnaturalización de la proteína proveniente de suero lácteo.

INTRODUCTION

In prior research, we addressed problems of poor leather quality by utilization of fillers produced from enzymatically modified waste proteins, specifically proteins from the leather and dairy industry (low quality gelatins and caseins or whey).[1-5] We demonstrated that these biopolymer products were indeed effective in improving subjective properties, such as handle, fullness, break and color. We addressed the problem of loose grain; "spring break" hides[6] and found that, after treatment, more cutting area was presented because of improvements in quality. In a more recent study,[7] we applied low molecular weight enzymatically-modified gelatin/WPI fillers to chrome-free or "wet white" leather. The chrome-free leather has been described as being flat, tinny, weak and/or having a poor break, requiring significant filling, utilizing heavy amounts of resins, polymers, and syntans. After treatment with the biopolymers, the leather was subsequently evaluated, and as seen in previous studies, the mechanical properties of the treated chrome-free leather were not significantly different from controls, but there was an improvement in the subjective properties, specifically fullness.

In this present study, we explored whether we could further improve subjective properties by utilization of high viscosity, high molecular weight products. Because of increasing cost of WPI, we decided to substitute lower cost WPC; to further reduce costs we replaced gelatin as the major protein with WPC. Historically, in preparation for enzyme modification of whey proteins, DTT has been used to reduce disulfide bonds and make the protein structures more open. A study has suggested that the relatively inexpensive $NaHSO_3$ would be effective as a substitute for DTT in opening up the whey.[8] In this present study, products were prepared using bisulfite and physical properties of the biopolymer compounds and their molecular weight distributions were determined. These products were applied to chrome-free leather, which was subsequently brought to crust. Subjective properties and mechanical properties were determined. Analyses of the products and the crust leather will be presented.

EXPERIMENTAL

Materials

Activa TG-TI, a microbial transglutaminase (mTgase) (approximately 100 units/g) containing maltodextrin as a carrier, with activity from pH 4.0 to 9.0, at 0 to 70°C, was obtained from Ajinomoto USA, Inc. (Paramus, NJ), stored at 4°C in a sealed package, and used as received. Commercial Type B gelatin from bovine skin, characterized in this laboratory as 175 g Bloom, was obtained from Fisher (Fairlawn, NJ). $NaHSO_3$ was obtained from Eastman Kodak Company (Rochester, NY). Dithiothreitol (DTT) was obtained from Sigma (St. Louis, MO). Whey protein concentrate containing 80% protein, (Hilmar™ 8000) was generously supplied by Hilmar Ingredients (Hilmar, CA). Chrome-free stock (upholstery weight) was purchased from a local tannery; area pieces (butt, belly and neck) and panels were sampled from this stock. All other chemicals were analytical grade.

Preparation of WPC/gelatin biopolymers:

Samples were prepared using varying concentrations of $NaHSO_3$ as follows. Whey protein concentrate (WPC) in combination with gelatin (175 Bloom) was suspended in water and allowed to swell for 2 h at room temperature (RT) and then stored overnight at 4°C. The samples were placed in a water bath at 65°C until dissolved. The pH was adjusted to 7.0-7.5 with 1 N NaOH. In the first set of experiments, the $NaHSO_3$ concentration was kept constant and added at 0.1% level; the resulting mixtures were heated at 38°C for 1 h. Samples without added enzyme or $NaHSO_3$, were run as controls. MTgase (calculated to be 0 to 10 units/g of total protein for biopolymer reactions) was prepared in 10 ml of water. In the second set of experiments, the bisulfite concentration varied from 0-1%, and mTgase was kept constant at 10 units/g of total protein. In each series of experiments, the enzyme solutions were added with stirring to the protein solutions to give final protein concentrations of 10% w/w for WPC and 1% w/v for gelatin.

In preparation of high molecular weight biopolymers, whey protein concentrate (WPC) in combination with gelatin (175 Bloom) was suspended in water and allowed to swell for about 2 h at RT and then stored overnight at 4°C. The samples were placed in a bath at 65°C until dissolved and the pH was adjusted to 7.0-7.5 with 1 N NaOH. In Test 1, 0.2% $NaHSO_3$ was added to protein solution and in Test 2, 0.1 % $NaHSO_3$ was added; both samples were heated at 38°C for 1 h. To Test 1, 5 units of mTgase (calculated to be 5 units/g of total protein for biopolymer reactions) was added to the protein solutions, whereas in Test 2, 3 units of mTgase was added. In both samples the enzyme was prepared in 50 ml of water to give a final protein concentration of 10% w/w for WPC and 2% w/v for gelatin.

Aliquots (10 ml) of the reaction mixtures described above (varying enzyme concentration, varying bisulfite concentration, preparation of high molecular weight products), were added to test tubes for melting point determination and 30-ml aliquots were poured into appropriate containers for determining gel strength. The samples were warmed to 50°C in a shaker bath and the reactions were carried out for 4 h. The enzyme was inactivated by heating the reaction products at 90°C for 10 min. The samples were cooled to room temperature and then chilled for 17 h at 10°C in a constant temperature bath. Physical analyses (gel strength, melting point and viscosity) were run on these samples. Aliquots of the samples were lyophilized and molecular weight distribution was determined by SDS PAGE. Sodium azide (70 µl of 1% solution) was added to the remaining solutions as a preservative and the samples were stored at 4°C.

Application of biopolymer to chrome-free leather (Area Samples and Panels)

Chrome-free stock for area study (six pieces, two pieces each from the butt, belly and neck area, three pieces /drum, ~100 g each), and panels of chrome-free stock (one panel/drum, ~3000 g each), were divided into tests and controls, the area pieces were placed in two small Dose drums (Model PFI 300-34, Dose Maschinenbau GmbH, Lichtenau, Germany), the panels in a larger Dose drum (VGI 1200 by 400), washed (400% float based on hide weight) by drumming for 30 min at 50°C, drained and refloated in sodium bicarbonate (1% on hide weight in 400% float). The samples were drummed at ambient temperature (25-28°C) until the pH stabilized. The floats were drained, the control samples set aside, and to the test samples, mTgase (5% based on hide weight) and the biopolymer (10% WPC and 2% gelatin, based on hide weight) were added. The samples were then drummed for 1 h at ambient temperature and then for 4 h at 50°C. The floats were drained and the samples were washed twice for 10 min at 50°C (400% float), drained, patted dry, and stored at 4°C. The tests and controls were retanned, colored and fatliquored (RCF) using an appropriate chrome-free formula as described in prior publication.[7] When completed, area samples and panels were toggled stretched and left to dry at ambient temperature and humidity. The samples were rewet, covered with plastic for two hours, then staked twice, and milled for approximately 16-18 h. No finishing operations were done to the hides and they were kept on a shelf in the conditioning room, at 20°C and 65% relative humidity for at least 3 days.

Analyses

Physical properties, molecular weight distribution, and mechanical properties.

Gel strength, melting point, viscosity, and molecular weight distribution (by SDS-PAGE) of the enzyme-treated proteinaceous solutions were determined as described in previous publications.[9,10] Mechanical properties (tensile strength, elongation, Young's Modulus, toughness index, tear strength, and thickness) were determined as described in a previous paper.[11]

Subjective evaluation RCF leather

Each treated and control sample was evaluated with respect to handle, fullness, grain (break) and color. A value from 1 to 5 was assigned for each parameter, with 1 being the worst and 5 being the best. From these ratings, an overall evaluation was determined and this value (from 1 to 5) was reported.

Yellowing Test

Two three-inch (76 mm) square pieces were cut from each of the treated and control samples. One square of each sample was placed in an oven, at 120 °C, for 72 h. After this time period, the heated samples were then compared to the unheated samples and evaluated with respect to color change. They were rated on a scale of 1 to 5, with 1 being the worst (highest color change) and 5 being the best (least effect on color).

RESULTS AND DISCUSSION

Preparation of WPC/gelatin biopolymers utilizing $NaHSO_3$

Previous studies have shown that modification of gelatin/WPI with enzyme could produce biopolymers with varying physical properties, the properties of which would make them amenable to be used as fillers.[1-5] Recently WPI has become increasingly expensive and we decided to substitute the more economical WPC for WPI. This product has a lower protein content (~80% as compared to WPI's ~92%), but when WPC in combination with gelatin was modified and physical properties compared to those of WPI with gelatin the physical properties are not significantly different (Table 1). To further make the process more economical, we substituted WPC for gelatin as the major protein (Table 1). All subsequent experiments utilized this latter combination.

TABLE 1
Treatment of gelatin, WPI and WPC with mTgase[a] Effect on physical properties

	Gel Strength (grams)	MP (°C)	Viscosity @ 60°C (cP)
Gel/WPI[b](0u)	340.5	36.4	7.7
Gel/WPI[b](1u)	364.5	36.6	8.7
Gel/WPC[c](0u)	382.7	35.8	6.8
Gel/WPC[c](1u)	380.5	36.4	7.5
WPC/gel[d](0u)	na	29.8	2.28
WPC/gel[d](1u)	na	30.0	3.37

[a]pH 7.0-7.5, 0.5% DTT @38°C for 1 h, 50°C for 4h.
[b]Concentration in solution: Gelatin = 10% w/w; WPI =2% w/v.
[c]Concentration in solution: Gelatin = 10% w/w; WPC =2% w/v.
[d]Concentration in solution: WPC = 10%; Gelatin = 1%.

In prior experiments in which whey was utilized in the biopolymers, DTT was employed to denature the protein. DTT is expensive so we investigated whether $NaHSO_3$, a more economical reductant, could be substituted. There has been a previously reported study successfully using $NaHSO_3$ [8] to denature whey prior to mTgase modification, however, this study did not look at the combination of whey with gelatin. In the following experiments we examined the influence of varying bisulfite and enzyme concentration on the physical properties of WPC/gelatin biopolymer products.

We found, when using a 10% w/w concentration of WPC and 1% w/v concentration of gelatin, that by increasing the units of enzyme (0-10 u) while keeping the bisulfite concentration constant (0.1%), there was no significant change in the gel strength (Figure 1a), a slight increase in melting point (from 29.0°C to 30.5°C) (Figure 1b), and a modest increase in viscosity (from 1.64 cP to 6.73 cP @ 60°C) (Figure 1c).

In Figure 2 the effect of increasing enzyme concentration on the molecular weight distribution is shown, and it appears that the bands representing the WPC (14,700, 18,300, and-35,000-40,000 Da) decrease in intensity as the enzyme concentration is increased. Furthermore, with increasing enzyme concentration, the band that does not enter the gel (above 200,000 Da) increases in intensity. Enzyme concentrations, 1a and 1b (Figure 2), indicate the absence and presence of bisulfite. The molecular weight distribution data corroborates the physical property data.

In the next experiment, 10 units of enzyme was used to modify the proteins (10% w/w WPC and 1% gelatin), while 0- 1% bisulfite was added to denature the whey protein. Increasing bisulfite concentration from 0 to 1% caused the gel strength to increase from 13.0 to 87.0 g (Figure 3a), above 0.2% bisulfite addition, the samples no longer melted at 60 °C, and the viscosity could not be determined (Figure 3b). Moreover, in this experiment we could see the effect of aging on a WPC/ gelatin enzyme-modified product, particularly in samples treated with 0.1 and 0.2% bisulfite additions. The melting points, which were read immediately, were around 33 °C, representing an increase of four degrees over the control sample, but the viscosity, typically measured after the melting points but read at a later date, could not be determined successfully because the sample had gelled irreversibly. This phenomenon of a sample no longer melting was seen in our earlier studies and this inability to melt suggested the existence of an initial gelled network that became more stable with time.[4,5]

The effect of increasing bisulfite concentrations on the molecular weight distribution is shown (Figure 3c) and as one progresses from 0 to 1%, the bands indicative of WPC (14,300, 18,700, and 35,000-40,000 Da) are decreasing in intensity. At the 0.1% concentration, a high molecular weight band that

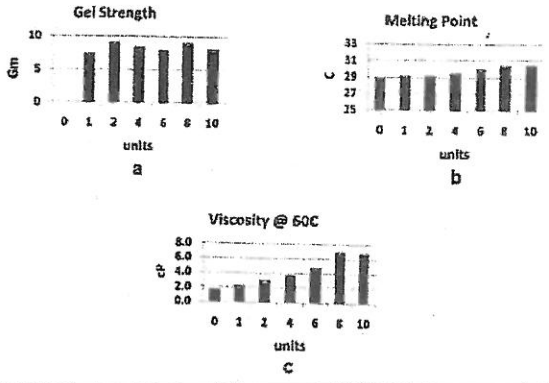

Figure 1. WPC (10% w/w) and 175-Bloom gelatin (1% w/v), treated with $NaHSO_3$ (0.1%) and mTgase (0-10 u/gm protein) @ pH 7.0-7.5, 50°C for 4 h; gel strength (a), melting point (b), and viscosity @ 60°C (c).

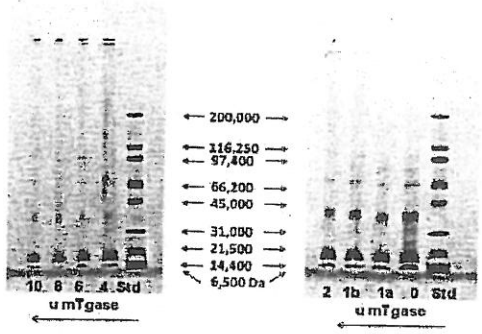

Figure 2. SDS-PAGE of WPC (10% w/w) and 175-Bloom gelatin (1% w/v) treated with $NaHSO_3$ (0.1%) and mTgase (0-10 u/gm protein) @ pH 7.0-7.5, 50°C for 4 h; lanes with 0 and 1a units of mTgase are controls, no NaHSO3 added; molecular weights are shown in Da.

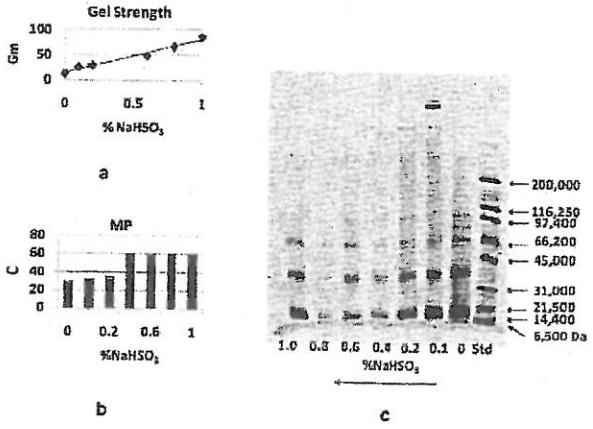

Figure 3. WPC (10% w/w) and 175 Bloom gelatin (1% w/v), treated with $NaHSO_3$ (0 to 1%) and mTgase (10 u/gm protein) @ pH 7.0-7.5, 50°C for 4 h; gel strength (a), melting point (b), and SDS-PAGE (c), molecular weights are shown in Da (c).

does not enter the gel appears, but is not seen in those with higher concentrations of bisulfite. These data are showing that the relatively inexpensive $NaHSO_3$ can be substituted for DTT in the WPC/gelatin systems to give products with varying properties that could be applicable to leather processing.

Preparation of biopolymers to treat chrome-free leather

Utilizing the information found in the above $NaHSO_3$ study, the following experiments were designed. WPC/gelatin biopolymers, using $NaHSO_3$, were prepared for evaluation as fillers for chrome-free leather. In one set of experiments, the 10% WPC/2% gelatin was treated with 0.2% $NaHSO_3$ and 5 units of enzyme (Test 1) and in the other, 10% WPC/2% gelatin was treated with 0.1% $NaHSO_3$ and 3 units of enzyme (Test 2).

TABLE 2
Effect of $NaHSO_3$ and enzyme concentrations on physical properties

	Gel Strength (grams)	MP (°C)	Viscosity (cP)
10%/2% WPC/gel (0.2%/5u)[a,c]	29.3	>60	na
10%/2% WPC/gel (0.1%/3u)[b,c]	22.0	33.0	4.89

[a]0.2% $NaHSO_3$ @ 38°C for 1h, 5 u mTgase.
[b]0.1% $NaHSO_3$ @ 38°C for 1h, 3 u mTgase.
[c]pH 7.0-7.5, 50°C, 4h.

Physical properties (Table 2) show that the former modification gave a product that did not melt at 60 °C and whose viscosity could not be determined. The latter product had similar properties to the low molecular weight products we had seen in earlier studies. The former product was obviously more robust (so robust that it had to be emulsified before application to leather). Wet white area pieces were treated with the two products; they were brought to the crust, evaluated and analyzed. It was found (Figure 4) that the high molecular weight product (Test 1) showed a significant improvement in fullness, break, and overall rating over the lower molecular weight product (Test 2). A series of experiments, applying this former (Test 1) product to area pieces and panels were run and mechanical properties, subjective evaluations and yellowing tests were carried out on crust leather.

Mechanical properties, subjective evaluation and yellowing test

After treatment and RCF of chrome-free leather with high molecular weight biopolymer, mechanical property determinations were carried out on the crust. The mechanical properties of those pieces cut from the butt, belly and neck areas are shown in Figure 5.

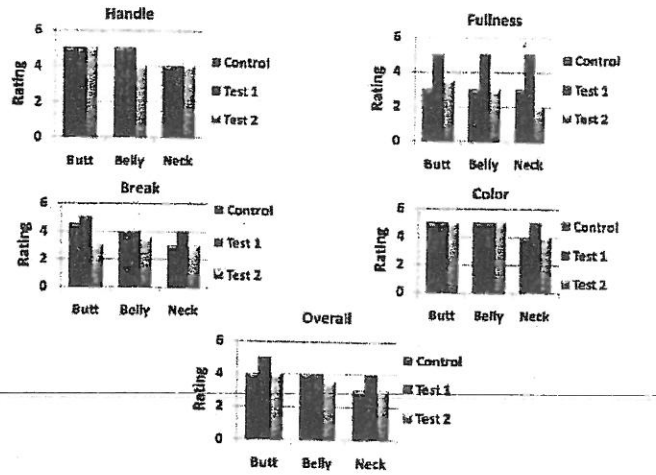

Figure 4. Subjective evaluation (handle, fullness, break, and overall) using rating scale of 1 = worst to 5 = best, of chrome-free stock (area pieces), treated with pH-adjusting agents alone (controls) and with mTgase and high (Test 1) and low (Test 2) molecular weight WPC/gelatin biopolymers, then RCF.

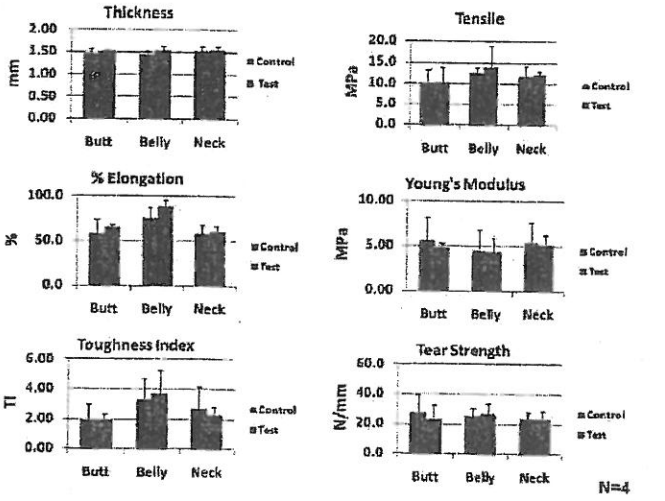

Figure 5. Mechanical properties (with STD Dev) of area pieces of chrome-free stock, treated with pH-adjusting agents alone (controls) and with mTgase and WPC/gelatin biopolymer (tests), then RCF; data are averaged from four trials.

As indicated by the error bars, there are no significant differences between the tests and the controls in any of the properties measured. When these data are averaged (Figure 6) with the data from the panel studies, the same trend is seen—no significant differences are indicated. For the most part, this is what we have seen in prior studies.

With respect to subjective evaluation of the leather treated with biopolymer, the results can be seen in Figure 7. Also shown is a comparison to leather treated with a lower molecular weight WPC/gelatin product. With regard to handle, no differences are discerned between tests and controls

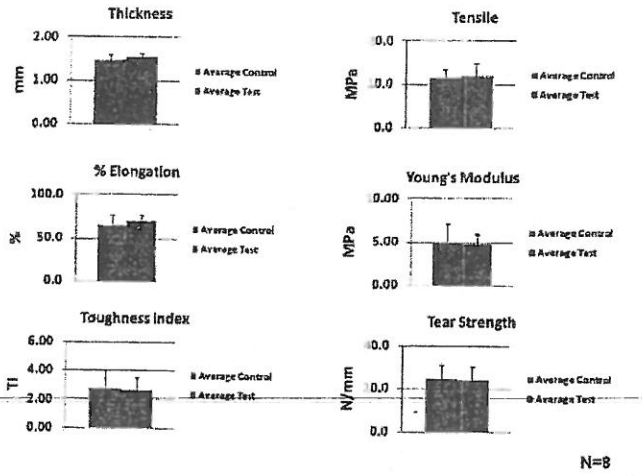

Figure 6. Mechanical properties (with STD Dev) of area pieces and panels of chrome-free stock, treated with pH-adjusting agents alone (controls) and with mTgase and WPC/gelatin biopolymer (tests), then RCF; data are averaged from eight trials.

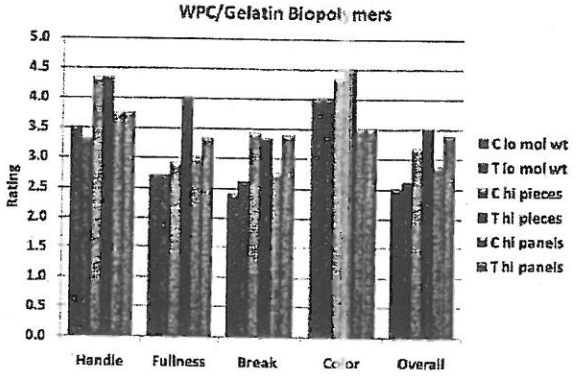

Figure 7. Subjective evaluation (handle, fullness, break, and overall) using rating scale of 1 = worst to 5 = best, of chrome-free stock (area pieces and panels), treated with pH-adjusting agents alone (controls) and with mTgase and low (n=5, area pieces) and high molecular weight (n=4, area pieces, and n=4, panels) WPC/gelatin biopolymers (tests), then RCF.

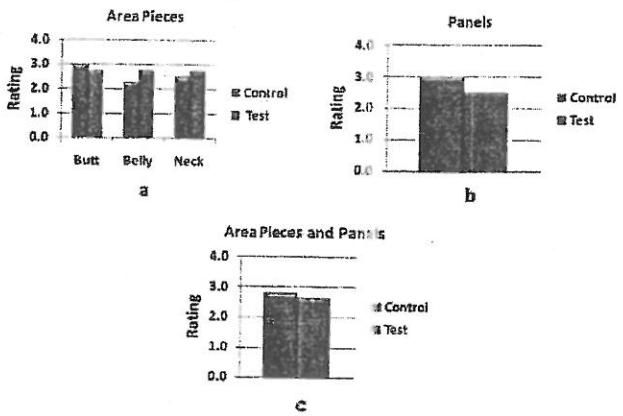

Figure 8. Yellowing test of chrome-free, area pieces (a) (n=4), panels (b) (n=4), and average of both (c); controls (treated with pH-adjusting agents alone) and tests (treated with WPC/gelatin), then RCF.

of high molecular weight treatments; in the low molecular weight treatments, there was a slight decrease in the test rating compared to control. With respect to fullness, the greatest improvement using high molecular weight polymers can be seen in both the pieces and panels within the set and over low molecular weight samples. The most dramatic improvement in break can be seen in panels from the high molecular weight biopolymer treatment. Overall, both pieces from the butt, belly and neck areas, and panels showed an improvement over controls in high molecular weight biopolymer treatments.

The results of yellowing tests on samples from biopolymer treatments can be seen in Figure 8. When individual pieces were evaluated (Figure 8a), there was a slight improvement in the control sample from the butt area, but in the belly and neck samples, the test samples fared better. When panels were evaluated (Figure 8b) and when all evaluations were averaged (Figure 8c), the control samples were slightly more resistant to yellowing. Protein fillers have been known to have an adverse effect on the yellowing test, however these samples performed rather well, showing little deterioration (compared to control samples).

CONCLUSIONS

$NaHSO_3$ can be substituted for DTT to denature or (cleave or reduce) disulfide bridges in whey and the preparation of a range of products using bisulphite, with varying physical properties can be achieved. Utilizing this technique, a high molecular weight biopolymer, consisting of mTgase-modified WPC/gelatin was prepared. The biopolymer product does not melt at 60 °C and as a result, viscosity cannot be determined; furthermore, SDS-PAGE gel of the modified product shows, when compared to untreated control, the characteristics of the gel have changed dramatically, with the profile normally seen for WPC altered, indicating a high degree of crosslinking. Next the biopolymer was tested on chrome-free leather. Subjective evaluation of the treated, RCF chrome-free, when compared to control samples and to samples treated with low molecular products, shows improvements in fullness, break and overall. There are no significant differences in mechanical properties, as well as no significant differences in yellowing test for treated samples, which is an improvement over other reported protein treatments.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the assistance of Nick Latona, Renée (Wildermuth) Latona and C.-K. Liu.

REFERENCES

1. Taylor, M. M., Marmer, W. N., and Brown, E. M.; Preparation and characterization of biopolymers derived from enzymatically modified gelatin and whey. *JALCA* **101**, 235-248, 2006.
2. Taylor, M. M., Marmer, W. N., and Brown, E. M.; Evaluation of polymers prepared from gelatin and casein or whey as potential fillers. *JALCA* **102**, 111-120, 2007.
3. Taylor, M.M., Marmer, W.N., and Brown, E.M.; Effect of fillers from enzymatically modified proteins on mechanical properties of leather. *JALCA* **103**, 128-137, 2008.
4. Hernàndez-Balada, E., Taylor, M.M., Phillips, J.G., Marmer, W.N., and Brown, E.M.; Properties of biopolymers produced by transglutaminase treatment of whey protein isolate and gelatin. *Bioresource Technology* **100**, 3638-3643, 2009.
5. Hernàndez-Balada, E., Taylor, M.M., Brown, E.M., Liu, C.-K., and Cot, J.; Whey protein isolate: A potential filler for the leather industry. *JALCA* **104**, 122-130, 2009.
6. Taylor, M.M., Lee, J. Bumanlag, L.P., Hernàndez Balada, E., Cooke, P.H. and Brown. E.M.; Treatment of low-quality hides with fillers produced from sustainable resources. Effect on properties of leather. *JALCA* **104**, 324-334, 2009.
7. Taylor, M.M., Lee, J., Bumanlag, L.P., Hernàndez-Balada, E., and Brown, E.M.; Treatments to enhance properties of chrome-free (wet white) leather. *JALCA* **106**, 35-43, 2011.
8. Wilcox, C. P., Clare, D.A., Valentine, V.W. and Swaisgood, H.E.; Immobilization and utilization of the recombinant fusion proteins trypsin-streptavidin and streptavidin-transglutaminase for modification of whey protein isolate functionality. *J. Agric. Food Chem.* **50**, 3723-3730. (2002).
9. Taylor, M. M., Cabeza, L. F., Marmer, W. N., and Brown, E. M.; Enzymatic modification of hydrolysis products from collagen using a microbial transglutaminase. I. Physical Properties. *JALCA* **96**, 319-332, 2001.
10. Taylor, M. M., Marmer, W. N., and Brown, E. M.; Molecular weight distribution and functional properties of enzymatically modified commercial and experimental gelatins. *JALCA* **99**, 129-140, 2004.
11. Liu, C.-K., and McClintick, M. D.; Measurements of the initial strain energy of leather. *JALCA* **92**, 157-171, 1997.